withstand the drag executed when the particle-target cell complex is exposed to a magnetic field. The particle-target cell complex will appear in the microscope as a "rosette", and two rosettes seen is enough to diagnose, for example, a cancerous condition.

Surprisingly, the present inventors were able to provide for binding strength of target cell-particle rosettes sufficient to withstand the drag executed when the target cell-particle complex is exposed to a magnetic field, yet sensitive and specific enough to detect down to one target cell per millions of non-target cells. As is known in the art, higher incubation temperatures produce stronger antibody-antigen binding, however higher temperatures also yield unspecified binding to non-target cells. As such, prior to the present method, one could not achieve a combination of sufficient binding strength and high sensitivity and specificity. Accordingly, the presently claimed invention which allows for sufficient binding to occur to form a target cell-particle rosette at an incubation temperature of about 4°C was quite surprising and not predictable by the art available at the time of the invention.

None of the references cited by the Examiner could have inspired Applicants to invent the presently claimed method. The cited references all have serious drawbacks and are drawn to such different methods that the Applicants would not have used any teachings of the reference for their present purpose.

The advantages of the presently claimed invention are illustrated in the enclosed descriptions from Labsoft (Cell Select, Separation of human epithelial cells) and the abstract by C. Siewert et al (A new rapid assay for combined immunomagnetic enrichment and immunocytochemical detection of disseminated tumor cells from PBMC).

With respect to the specific rejections of the Examiner, Applicants submit the following remarks.

Widder in view of Connelly

Claims 22-25, 28, 29, 33, and 105 have been rejected under 35 U.S.C. 103(a) as allegedly being obvious over Wider et al. in view of Connelly et al. Applicants respectfully traverse this rejection.

Widder et al. fail to teach detection of a specific target cell as claimed in the present application. *Widder et al.* disclose a method for coarse separation of blood cells, not detection of individual target cells. For example, see the last line of page 11 of *Widder*, where it is disclosed that the

specificity of the *Widder* method is greater than 10 percent. Such a poor specificity is not capable of detecting a specific target cell. The Examiner indicated that Applicants failed to provide data to support allegations of the lack of specificity of the method of *Widder* relative to the presently claimed invention. Applicants respectfully assert that such a showing of data is not required because *Widder* discloses a specificity that is clearly unacceptable for the presently claimed invention.

Furthermore, due to the use of protein A, the microspheres of *Widder* will adhere to non-target cells and target cells alike and cause unacceptable reduction in specificity. In addition to binding the Fc portion of IgG cells, protein A will also bind to B cells and plasma cells present in a sample. Clearly, the method of *Widder* would involve non-specific binding if the target cells lacked a molecule capable of binding Protein A and the mixed cell sample contained cells capable of binding Protein A. In summary, *Widder* cannot achieve the present invention, since this method cannot detect a specific target cell in a cell suspension. Further, there is no teaching or suggestion in *Widder* of forming target cell-bead rosettes as claimed in the present application which can then be quantified.

Connelly does not overcome the deficiencies of *Widder*, as *Connelly* also fails to teach or suggest a method of detecting specific target cells in a cell suspension. Additionally, *Connelly* teaches a cell fixative composition for fixing the internal components of a cell without disrupting the cell surface components. *Connelly* uses fixatives without destroying the cells structurally, but the cells are killed. The present invention examines live cells, and these cells can be viewed in a microscope without using fixatives at all. *Connelly* does not address detection methods for target cells as presently claimed.

Therefore, even if one of skill in the art were to combine the teachings of *Widder* and *Connelly*, he or she would not achieve the present invention. The references do not teach or suggest, alone or in combination, a method for detecting a specific target cell in a cell suspension, wherein beads are coated with an agent directed against specific membrane structures expressed only on the target cells and not on non-target cells, and wherein target cell-bead rosettes are formed and quantitated as described and claimed in the present application.

Withdrawal of the rejection is respectfully requested.

Widder in view of Kemmer, Holmes, and Terasaki

Claims 22, 34-40, 43, 48, 67, 69, 71, 72, 75, 87-89, 92, 93, 96, 101 have been rejected under 35 U.S.C. 103(a) as allegedly being obvious over Wider et al. in view of Kemmer et al. and Holmes et al. and in further view of Terasaki et al. Applicants respectfully traverse this rejection.

As discussed above *Widder* does not teach or suggest detection of a specific target cell as claimed in the present application. *Kemmer*, *Holmes* and *Terasaki* fail to cure the deficiencies of *Widder*.

Kemmer and *Holmes* each teach non-specific methods, while *Terasaki* teaches hybridoma cell lines. None of the references, alone or in combination teach or suggest a method for detecting a specific target cell using the presently claimed method.

Kemmer teaches the use of beads for enriching a cell population prepared from a solid tumor containing mainly tumor cells, and the reference uses the bead/cell complex to assess the effects of the enrichment. However, despite the high number of malignant cells in relation to normal cells in the reference, the results of the method provided heterogenic cells, including tumor cells. In fact, only 96% of the bead-rosetting cells with the specific antibody proved to be tumor cells and 5% of the cells attached to the beads coated only with an irrelevant antibody bound to tumor cells (see page 199, column 1 of the reference). Moreover, of the 34% cells that bound control beads coated with an antibody recognizing the human leukocyte common antigen (Dako-LC), as much as 35% turned out to be tumor cells (see page 199, column 1 of the reference). This is a good example of the unspecificity of *Kemmer's* method since leukocyte common antigen is not expressed on the tumor cells. These data demonstrate a highly unspecific method which cannot be used to specifically and reliably detect a specific target cell in a mixed cell population.

Holmes also provides a nonspecific method for separating haemopoietic progenitor cells from a mixed population of haemopoietic cells. *Holmes* provides a combination of positive and negative selection wherein both procedures can be performed in opposite succession and the beads are detached and removed from the cells. The method is performed with haemopoietic cells, and there is no required specificity. The positive selection antibody is taught as being reactive with a broadly expressed HPC antigen, e.g., an antibody reactive with the CD34 antigen. *Holmes* goes on to state that, "[M]ore broadly active antibodies are also of value since the negative selection step will remove unwanted cells included within the wider antigen groupings and leave only the desired HPC" (see page 5, lines 17-23). Other examples of positive selection

antibodies include a pan class II specific IgM antibody, and an IgG which also recognizes a monomorphic DR antigen on stem cells. Similarly, the negative selection antibody is nonspecific. Thus, *Holmes* provides a nonspecific method that uses antibodies found on a variety of cell types. There is no teaching or suggestion of a method of detecting a specific target cell as claimed in the present application.

Terasaki teaches using hybridoma cell lines for producing antibodies for detection of an epitope found in neoplastic cells, as well as a significant number of normal tissues (see column 2), and does not refer to binding antibodies to cells. Further, *Terasaki* teaches detection of free antigen in the blood. In this case, all cells are removed from the sample. Therefore, *Terasaki* adds nothing to the other references in this case.

Additionally, there is no reasonable expectation of success when modifying or combining the references discussed above. *At best*, one of skill in the art might expect to achieve a method with the level of non-specificity as taught in *Widder*. In such a method, non-target-cells would be included in the rosettes and the method would not be useful as a diagnostic measure. In *Holmes*, the number of particles bound to non-target-cells would be the same as the number of particle bound to target-cells, because sub-groups of normal cells will express the target antigen on their surfaces. There is no reasonable expectation that a method of detecting a specific target cell can be achieved by combining/modifying the references.

Withdrawal of the rejection is respectfully requested.

Widder in view of Forrest

Claims 22, 46-48, 51, 59-62, 64, 66, 67, 69, 71, 78, 79, 106, and 107 have been rejected under 35 U.S.C. 103(a) as allegedly being obvious over Wider et al. in view of Forrest et al. Applicants respectfully traverse this rejection.

As discussed above *Widder* does not teach or suggest detection of a specific target cell as claimed in the present application. The combination of Forrest with Widder does not overcome the deficiencies of Widder.

The Examiner stated that it would have been obvious to one of ordinary skill in the art to use a binding system such as avidin-biotin as taught by *Forrest et al.* in the method of *Widder et al.* because *Forrest et al.* teach that avidin-biotin provides a very rapid and high binding affinity which offers the advantage of a more accurate and rapid assay. However, there is no teaching or

suggestion of the presently claimed method, i.e., a method of detecting a specific target cell in a cell suspension using paramagnetic particles or beads coated with a monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell and not on a non-target-cell in the cell suspension, forming target cell-bead rosettes, and quantitating the target cell-bead rosettes. More specifically, there is no teaching or suggestion that incubation at about 4°C can produce adequately strong binding to allow for quantification of target-cell bead rosettes. There is no teaching or suggestion of the highly specific detection method presently claimed.

Withdrawal of the rejection is respectfully requested.

Jensen in view of Hermentin or Ullman

Claims 22-25, 28-29, 33-40, 43, 46-48, 51, 59-62, 64, 66-67, 69, 71-72, 75, 78-79, 87-89, 92-93, 96, 101, and 105-107 have been rejected under 35 U.S.C. 103(a) as allegedly being obvious over Jensen taken together with Hermentin et al. or Ullman et al. Applicants respectfully traverse the rejection.

The method of *Jensen* neither has the sensitivity nor the specificity of the presently claimed method. In *Jensen*, if 95% of the target cells are removed from the cell population, all of the target cells are considered to be removed, whereas the present methods detect target cells comprising less than 0.1% of the population down to 2 target cells per 10^7 cells. The lack of sensitivity and specificity of the *Jenson* method is due to the incubation conditions of Jensen, typically 15-25°C (column 12, line 35). Such a temperature range is known in the art to give unspecified binding to non-target cells.

In contrast, the presently claimed method includes an incubation temperature of about 4°C. As it is well known that lower incubation temperatures produce weaker antibody-antigen binding, Applicants surprisingly found that adequately strong binding could occur at such incubation temperatures. Accordingly, *Jensen* does not teach or suggest a method where an incubation temperature of about 4°C provides sufficient binding strength, sensitivity, and specificity to detecting a specific target cell in a mixed cell suspension.

Hermentin or *Ullman* do not overcome the deficiencies of *Jensen*. Neither *Hermentin* nor *Ullman*, alone or in combination with *Jensen*, teach or suggest that sufficient binding can occur to form a target cell-bead rosette at an incubation temperature of about 4°C.

Additionally, one of skill in the art would not combine *Jensen* with *Hermentin* or *Ulman* to arrive at the presently claimed invention. The references disclose different methods with different purposes.

Hermentine discloses paramagnetic protein conjugates useful for the specific removal of cells from solutions or body fluids, wherein detergents have been added to prevent non-specific antibody adsorption onto paramagnetic particles (column 4, lines 45-48). This non-specific adsorption is not a factor in the present method since the particles are exposed to only the specific antibody which is to be adsorbed. The problem in the present method being addressed by the addition of mild detergents is the unspecified binding of the antibody-particle complex to non-target cells. Thus, the non-specific adsorption problem of *Hermentin* is quite different than the problem of the present method. Therefore, the purpose and teaching of mild detergents in *Hermentin* is completely unrelated to the present invention.

Ullman uses paramagnetic and nonmagnetic poarticles to obtain nonspecific aggregation of particles and uses detergent to dissolve the aggregates. In the presently claimed method, aggregation of particles is not desired, as it would destroy the method.

Withdrawal of the rejection is respectfully requested.

Double Patenting Rejection

Claims 22-23, 28-29, 33-40, 46-48, 51, 59-62, 64, 66-67, 69, 71-72, 75, 79, 87-89, 92-93, 96, 101, and 105-107 have been rejected under 35 U.S.C. 102(f) as allegedly being anticipated by Fodstat et al. (Application serial number 08/704,619). These claims have also been provisionally rejected under 35 U.S.C. 101 as allegedly claiming the same invention as that of claims 19-24, 26-40, 44, 45, 47-72, 74-89, 93-109, and 118-122 of copending Application No. 08/704,619, and have been provisionally rejected under 35 U.S.C. 102(e) as allegedly being anticipated by copending Application No. 08/704,619. Applicants respectfully traverse these rejections.

The instant application was filed September 10, 1993, while copending Application No. 08/704,619 was filed March 10, 1995 and claims priority to March 10, 1994. Because the instant application was filed before the priority date of copending Application No. 08/704,619, copending Application No. 08/704,619 cannot anticipate the instant application.

Additionally, unlike the present application, copending Application No. 08/704,619 claims a method that includes separating particle-target cell complexes from unbound particle, unspecifically bound non-target cells and unbound non-target cells in the mixture of coated paramagnetic particles and cell suspension by transferring the mixture to a separating apparatus. The claimed inventions are different. Accordingly, even if copending Application No. 08/704,619 could anticipate the instant application, it would not anticipate the instantly claimed invention.

Withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the remarks presented herein, Applicants respectfully submit that the claims are in condition for allowance. Notification to that effect is earnestly solicited. If prosecution of this case could be facilitated by a telephonic interview, the Examiner is encouraged to call the undersigned.

Respectfully Submitted,

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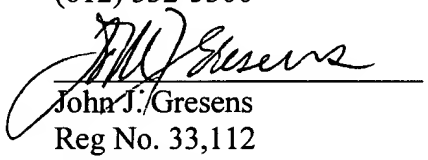
DATE: 6/27/2000



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A NEW RAPID ASSAY FOR COMBINED IMMUNOMAGNETIC ENRICHMENT AND IMMUNOCYTOCHEMICAL DETECTION OF DISSEMINATED TUMOR CELLS FROM PBMC

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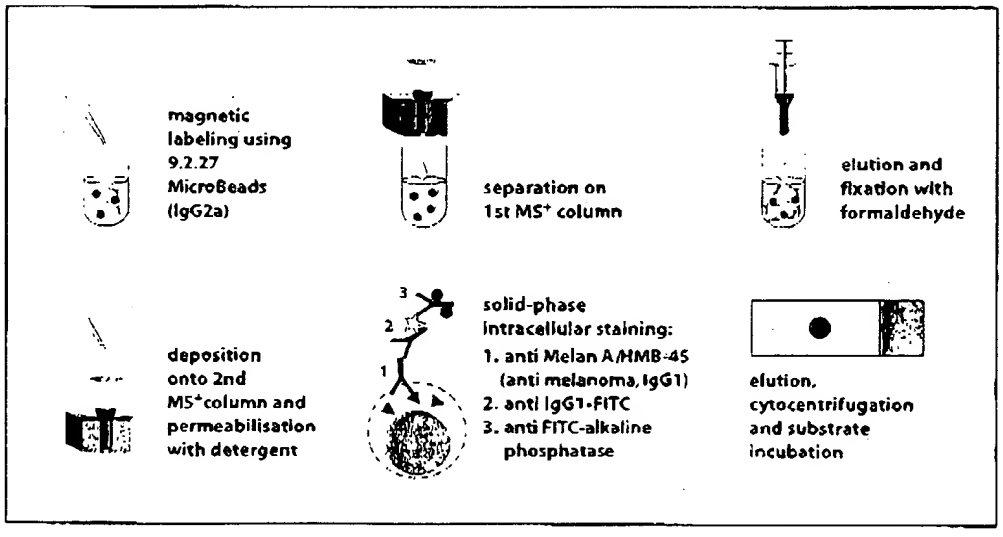
Abstract No. 3754

Detection of single circulating tumor cells in peripheral blood of patients with solid tumors is considered to be of great prognostic relevance for early micrometastatic disease. Commonly used methods, including RT-PCR and immunocytochemistry display only a limited sensitivity and specificity.

Here we describe a new assay that permits rapid and reliable detection of extremely rare disseminated tumor cells by a combination of magnetic cell sorting (MACS), "solid-phase" intracellular staining on columns and immunocytochemical detection on slides. The efficiency of the assay was demonstrated in a model system in which defined numbers of cells from a melanoma cell line were spiked to PBMC samples and feasibility was tested on clinical samples of peripheral blood from patients with stage III-IV malignant melanoma.

Immunomagnetic Enrichment and Immunocytochemical Staining of Melanoma Cells from PBMC



Melanoma cells are specifically magnetically labeled using MACS MicroBeads conjugated to an anti melanoma antibody (9.2.27) and enriched in a first round from PBMC on a high-gradient magnetic positive selection column. The enriched cell fraction is eluted and fixed with formaldehyde. The fixed cells are re-applied onto a second column. Whilst immobilised to the matrix of the magnetic column, melanoma cells are permeabilised and labeled with antibodies for the detection of intracellular antigens in a double indirect way (anti Melan A and HMB-45, anti isotyp-FITC, anti FITC-Alkaline Phosphatase). After completion of all staining steps, enriched cells are eluted and transfered to slides. Slides are finally developed using a substrate for alkaline phosphatase and analysed by light microscopy.

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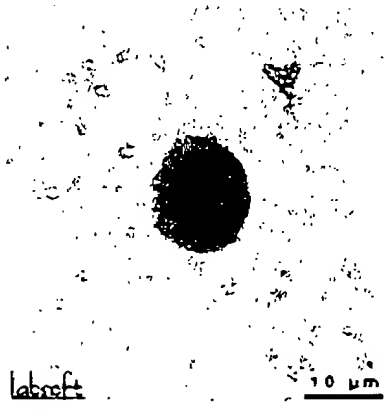
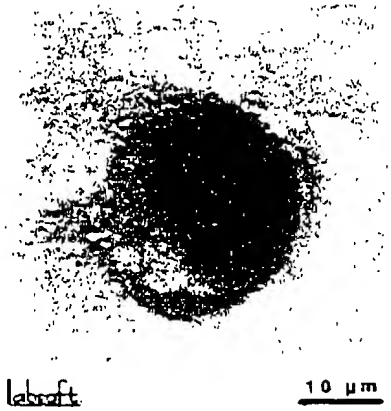
CellSelect

Separation of human epithelial cells

CellSelect is a separation technique based on magnetic beads to isolate cells out of various body fluids or tissue homogenates. CellSelect is using individual coated magnetic beads and buffer solutions developed in a co-operation with the chemicell GmbH. The matrix, the surface, the magnetic strength and the resultant physical and biological characteristics have been optimized. At the special modified surface of the magnetic beads antibodies will be coupled by Labsoft which allow the selective isolation of various cell species in high purity. With this beads pro- and eucaryotic cells can be isolated in living, undamaged condition to ensure the immunological and molecularbiological examination of this cells. Custom-designed requests can be considered with regard to the target cells or target antigenes, the sample material for the isolation and the optimization of the isolation methods including the modification of the beads and needed applications. The products, available in single components, include the magnetic beads, magnet racks and buffer solutions.



Pic. 1: isolated breast cancer cell (out of 18 ml patient blood) immunocytochemical stained (antipan CK FastRed, Hämatoxylin)



Pic. 2: isolated MCF-7 ceil (human breast adenocarcinoma, ATCC-number HTB 22, out of 9 ml blood) stained (toluidin blue).